

刺果番荔枝种子中的新环肽——刺果番荔枝环肽 A *

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摘要 从刺果番荔枝种子中得到 1 个新环肽, 命名为刺果番荔枝环肽 A (annomuricatin A)。通过多种 2D-NMR 技术、pos. FAB-MS 和氨基酸分析, 其结构确定为环(脯-苯丙-缬-丝-丙-甘), 是 1 个环六肽。

关键词 刺果番荔枝, 番荔枝科, 种子, 环肽, 刺果番荔枝环肽 A

ANNOMURICATIN A, A NEW CYCLOPEPTIDE FROM THE SEEDS OF ANNONA MURICATA

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Abstract A new cyclopeptide, annomuricatin A, has been isolated from the seeds of *Annona muricata* and the structure was elucidated by extensive 2D-NMR methods, pos. FAB-MS, and amino acid analysis.

Key words *Annona muricata*, Annonaceae, Seeds, Cyclopeptide, Annomuricatin A

Recently we have reported a series of cyclopeptides with unique structures from higher plants^[1-2]. As part of our on going investigation of cyclopeptides, we have isolated several cyclopeptides from Annonaceae for the first time^[3]. The fruits of *Annona muricata* Linn. (Annonaceae) is edible in Yunnan province(China). As well as others in the family Annonaceae it has recently come under intense scrutiny as potential sources of the potent biologically active Annonaceous acetogenins^[4-7]. Now we describe the isolation and structure determination of one novel cyclopeptide named annomuricatin A from the plant seeds based on extensive 2D-NMR methods, pos. FAB-MS, and amino acid analysis.

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RESULTS AND DISCUSSION

Annomuricatin A was isolated from the CHCl_3 fraction of the alcohol extract of *A. muricata* seeds by column chromatography as described in the experimental.

Annomuricatin A, needles, gave a negative ninhydrin reaction and showed a peak due to $[\text{M}+1]^+$ at m/z 559.2880(cald. 559.2949, $\Delta=6.8\text{mmu}$) corresponding to molecular formula: $\text{C}_{27}\text{H}_{38}\text{N}_6\text{O}_7$ in the positive HRFAB-MS. IR maxima absorptions at 3250, 1720, 1685 cm^{-1} and UV at 202(2.8×10^3) nm indicated the compound might be a peptide^[1]. Amino acid analysis of the compound after hydrolysis with 6 mol / L HCl at 110 °C gave the composition: Ser(1eq), Gly(1eq), Ala(1eq), Val(1eq), Phe(1eq), and Pro(1eq). Extensive application of 2D-NMR techniques was then used to determine the identity of the six amino acid units. The 400 MHz ^1H NMR spectrum clearly showed the presence of only five amide NH at δ 8.91, 8.23, 8.15, 8.05, 7.43, and the 100 MHz ^{13}C NMR spectrum showed six amide CO at δ 171.55, 170.54, 170.38, 169.62, 168.64, 167.89. By following the spin systems of these protons using $^1\text{H}-^1\text{H}$ COSY, $^{13}\text{C}-^1\text{H}$ COSY, COLOC spectra, these amino acids were determined to be serine, glycine, alanine, valine, phenylalanine, and proline units. The spectral data are shown in Table 1.

To further determine the sequence and the peptide to be a cyclopeptide, COLOC and pos. FAB-MS spectra were performed. The sequence of amino acid is summarized by COLOC spectra in Fig.1^[8]. In the positive FAB-MS spectrum the peptide gave $[\text{M}+1]^+$ at m/z 559, and the Mw was accorded with that of a cyclopeptide containing the amino acids mentioned above. The pathway is summarized by pos. FAB-MS in Fig.2. Therefore, the structure of the peptide named annomuricatin A, a hexacyclopeptide, was elucidated as cyclo-(prolyl-phenylalanyl-valyl-seryl-alanyl-glycyl).

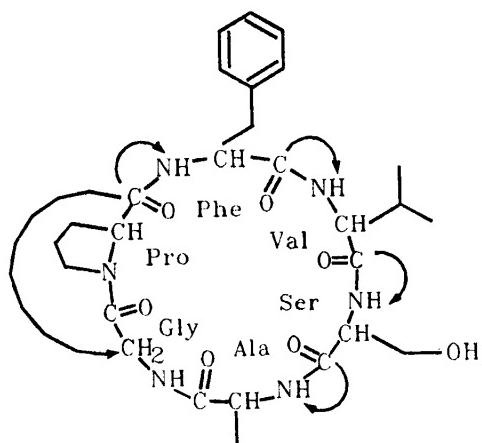


Fig. 1 Selected signals of COLOC spectra of
annomuricatin A

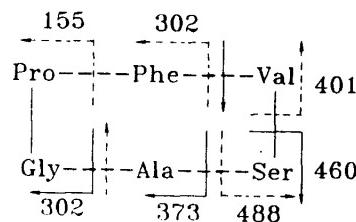


Fig. 2 Selected signals of pos. FAB-MS of
annomuricatin A

EXPERIMENTAL

Mp: uncorr. Optical rotation was recorded on a SEPA-300. UV was obtained on a UV-210. IR was recorded on a PE-577. Pos. FAB-MS were measured on a Autospec-3000. NMR were taken at a Bruker AM-400 in C₅D₅N soln using TMS as int. standard. Amino acid analysis was performed on a Hitachi 835-50.

Table 1 ¹H and ¹³C NMR spectral data of Annomuricatin A (in C₅D₅N, 400MHz for δ_H, 100MHz for δ_C, TMS)

Amino acid residues	H	C	Amino acid residues	H	C
Gly			Val		
α	3.11(1H, dd) 3.78(1H, m)	42.35	α	3.90(1H, t, 9.5)	62.14
NH	8.91(1H, dd, 4.0, 8.4)		β	2.05(1H, m)	29.20
C=O		171.55	γ	0.86(6H, 2×CH ₃)	19.14
Pro			NH	8.05(1H, d, 8.5)	19.27
α	4.14(1H, m)	61.24	C=O		170.54
β	1.83(1H, m) 2.05(1H, m)	28.49	Ser		
γ	2.05(2H, m)	25.08	α	4.29(1H, m)	54.76
δ	3.45(1H, m) 3.78(1H, m)	46.82	β	3.45(1H, m) 3.78(1H, m)	60.95
C=O		167.89	NH	8.15(1H, d, 9.2)	
Phe			C=O		168.64
α	4.60(1H, m)	53.67*	α	4.60(1H, m)	46.20*
β	2.62(1H, t) 3.26(1H, dd, 4.3, 13.0)	38.00	β	1.23(3H, d, 6.9)	17.30
φ	7.20(5H, m)	125.97 127.77 129.53 137.40	NH	7.43(1H, d, 7.3)	
NH	8.23(1H, d, 9.5)		C=O		170.38
C=O		169.62			

* The assignments may be reversed.

Extraction and isolation. Crushed air-dried seeds of *A. muricata* (2kg, cultivated in Xishuangbanna, Yunnan province in China) were repeatedly percolated with 95% EtOH and the extracts concd in vacuo. The EtOH extract was partitioned with CHCl₃ to yield the CHCl₃ soluble fraction which was then partitioned between petroleum ether and 90% aqueous MeOH (1:1) to yield the 90% aqueous MeOH soluble fraction(200g). The 90% aqueous MeOH fraction(160g) was subjected to column chromatography on silica gel using petroleum ether:EtOAc:MeOH gradient elution, affording annomuricatin A(105mg).

Annomuricatin A. Yield 6.6 × 10⁻³%, needles(MeOH), mp 285—287°C, [α]_D²³+11.28 ° (C₅H₅N; c 0.4). UVλ_{max}^{EtOH} nm(ε): 202(2.8 × 10³); IRν_{max}^{KBr} cm⁻¹: 3250, 1720, 1685. ¹H and ¹³C NMR see Table 1. Pos. FAB-MS m/z: 559[M+1]⁺(cald for C₂₇H₃₈N₆O₇, 559.2949, found 559.2880), 488, 460, 401, 373, 302, 155. Amino acid analysis (standard method): Ser(1eq), Gly(1eq), Ala(1eq), Val(1eq), Phe(1eq), and Pro(1eq).

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